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(71) Applicant(s)
Cambridge Antibody Technology Limited

(Incorporated in the United Kingdom)

The Science Park, Melbourne, ROYSTON, Cambs, SG8 6JJ, United Kingdom

(72) Inventor(s)
Julia Elizabeth Thompson
Tristan John Vaughan
Andrew James Williams
Jonathan Alexander Green
Ronald Henry Jackson
Louise Bacon
Kevin Stuart Johnson

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(72) cont
Alison Jane Wilton
Philip Ronald Tempest
Anthony Richard Pope

(74) Agent and/or Address for Service
Mewburn Ellis
York House, 23 Kingsway, LONDON, WC2B 6HP, United Kingdom

(54) Human antibodies specific for human transforming growth factor beta-1 and beta-2

(57) Human antibodies specific for human transforming growth factor-β (TGF-β), bind to TGF-β1 and/or TGF-β2 preferentially compared with TGF-β3 and are useful in the treatment of fibrotic and immune/inflammatory disease. A specifically disclosed antibody binds the active form of TGF-β2, neutralising its activity but does not bind the latent form.

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Figure 1(b)(i)

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            10          20          30          40
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT GTG TCT CTG GGC
 D   I   V   M   T   Q   S   P   D   S   L   A   V   S   L   G>

50          60          70          80          90
GAG AGG GCC ACC ATC AAC TGC AAG TCC AGC CAG AGT CTT TTA TAC AGC
 E   R   A   T   I   N   C   K   S   S   Q   S   L   L   Y   S>

  100         110         120         130         140
TAC AAC AAG ATG AAC TAC TTA GCT TGG TAC CAG CAG AAA CCA GGA CAG
 Y   N   K   M   N   Y   L   A   W   Y   Q   Q   K   P   G   Q>

    150         160         170         180         190
CCT CCT AAG CTG CTC ATT AAC TGG GCA TCT ACC CGG GAA TCC GGG GTC
 P   P   K   L   L   I   N   W   A   S   T   R   E   S   G   V>

       200         210         220         230         240
CCT GAC CGA TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC
 P   D   R   F   S   G   S   G   S   G   T   D   F   T   L   T>

           250         260         270         280
ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA GTT TAT TAC TGT CAG CAA
 I   S   S   L   Q   A   E   D   V   A   V   Y   Y   C   Q   Q>

290         300         310         320         330
TAT TAT GCA ACT CCT CTG ACG TTC GGC CAC GGG ACC AAG GTG GAA ATC
 Y   Y   A   T   P   L   T   F   G   H   G   T   K   V   E   I>

  340
AAA CGT
 K   R
```

Figure 1(b)(ii)

```
            10          20          30          40
CAC GTT ATA CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 H   V   I   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

50          60          70          80          90
ACA GTC AGG ATC ACG TGC CAA GGA GAC AGC CTC AAA AGC TAC TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   K   S   Y   Y   A>

  100         110         120         130         140
AGT TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

    150         160         170         180         190
GGT GAA AAC AGC CGG CCC TCC GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   E   N   S   R   P   S   G   I   P   D   R   F   S   G   S>

       200         210         220         230         240
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

           250         260         270         280
GAT GAA GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT GGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   G   T   H>

290         300         310         320         330
CTA GAA GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 L   E   V   F   G   G   G   T   K   L   T   V   L   G
```

Figure 19

(i)

```
                10                  20                  30                  40
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 E   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>

50                  60                  70                  80                  90
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>

  100                 110                 120                 130                 140
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>

    150                 160                 170                 180                 190
GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   W   Y   D   G   S   N   K   Y   Y   A   D   S   V>

        200                 210                 220                 230                 240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>

            250                 260                 270                 280
CTG CAA ATG GAC AGC CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   D   S   L   R   A   E   D   T   A   V   Y   Y   C>

290                 300                 310                 320                 330
GGA AGA ACG CTG GAG TCT AGT TTG TGG GGC CAA GGC ACC CTG GTC ACC
 G   R   T   L   E   S   S   L   W   G   Q   G   T   L   V   T>

  340
GTC TCC TCA
 V   S   S
```

Figure 19 (ii)

```
                10                  20                  30                  40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

50                  60                  70                  80                  90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>

  100                 110                 120                 130                 140
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

    150                 160                 170                 180                 190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   S   G   S>

        200                 210                 220                 230                 240
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

            250                 260                 270                 280
GAT GAG GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT AGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   S   T   H>

290                 300                 310                 320                 330
CGA GGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 R   G   V   F   G   G   G   T   K   L   T   V   L   G
```

Figure 19 (iii)

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          10          20          30          40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

50          60          70          80          90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>

 100         110         120         130         140
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

   150         160         170         180         190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC GCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   A   G   S>

      200         210         220         230         240
AAC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAG
 N   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

          250         260         270         280
GAT GAG GCT GAC TAT TAC TGT AGC TCC CGG GAC AGC AGT GGT AAC CAT
 D   E   A   D   Y   Y   C   S   S   R   D   S   S   G   N   H>

290         300         310         320
GTG GTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   V   F   G   G   G   T   K   L   T   V   L   G
```

Figure 19 (iv)

```
           10          20          30          40
GAT GTT GTG ATG ACT CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA
 D   V   V   M   T   Q   S   P   S   S   L   S   A   S   V   G>

50          60          70          80          90
GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGC ATT AGC AAT TAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y>

  100         110         120         130         140
TTA GCC TGG TAT CAG CAA AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
 L   A   W   Y   Q   Q   K   P   G   K   A   P   K   L   L   I>

    150         160         170         180         190
TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGT GGC
 Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>

       200         210         220         230         240
AGT GGA TCT GGG ACA GAA TTC ACT CTC ACA ATC AGC AGT CTG CAA CCT
 S   G   S   G   T   E   F   T   L   T   I   S   S   L   Q   P>

           250         260         270         280
GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCT CGA
 E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   R>

290         300         310         320
ACG TTC GGC CAA GGG ACC AAA GTG GAT ATC AAA CGT
 T   F   G   Q   G   T   K   V   D   I   K   R
```

CLAIMS:

1. A specific binding member comprising a human antibody antigen binding domain specific for human TGFβ which binds the human TGFβ isoforms TGFβ2, TGFβ1, or TGFβ2 and TGFβ1, preferentially over TGFβ3.

2. A specific binding member according to claim 1 which neutralises TGFβ2, TGFβ1, or TGFβ2 and TGFβ1.

3. A specific binding member according to claim 1 or claim 2 wherein said human antibody antigen binding domain is for the TGF-β isoform TGF-β2.

4. A specific binding member according to claim 3 wherein said human antibody antigen binding domain comprises a VH domain which has the amino acid sequence shown in Figure 2(a) (i) or Figure 2(a) (ii).

5. A specific binding member according to claim 3 or claim 4 wherein said human antibody antigen binding domain comprises a VL domain which has the amino acid sequence shown in any of Figures 2(b) (i) to (v)

6. A specific binding member according to claim 5 wherein said human antibody antigen binding domain comprises a pairing of a VH domain and a VL domain selected from:

(a) 6H1 VH, of which the amino acid sequence is shown

in Figure 2(a) (i), and 6B1 VL, of which the amino acid sequence is shown in Figure 2(b) (iii);

(b) 6H1 VH, of which the amino acid sequence is shown in Figure 2(a) (i), and 6H1, of which the amino acid sequence is shown in Figure 2(b) (i);

(c) 6H1 VH, of which the amino acid sequence is shown in Figure 2(a) (i), and 6A5 VL, of which the amino acid sequence is shown in Figure 2(b) (ii).

7. A specific binding member according to claim 6 wherein said human antibody antigen binding domain comprises the VH domain 6H1 VH, of which the amino acid sequence is shown in Figure 2(a) (i), and the VL domain 6B1 VL, of which the amino acid sequence is shown in Figure 2(b) (iii).

8. A specific binding member according to claim 3 wherein said human antibody antigen binding domain comprises a complementarity determining region (CDR) with an amino acid sequence identified as a CDR in any of the sequences shown in Figures 19 (i) to (iv).

9. A specific binding member according to claim 8 wherein said human antibody antigen binding domain comprises a VH domain which comprises a CDR3 with a sequence shown as CDR3 in Figure 19 (i).

10. A specific binding member according to claim 3

which competes for binding to TGF-β2 with a specific binding member according to claim 6.

11. A specific binding member according to claim 10 which competes for binding to TGF-β2 with a specific binding member according to claim 7.

12. A specific binding member according to claim 3 which binds the peptide TQHSRVLSLYNTIN.

13. A specific binding member according to claim 3 which binds the active form of TGFβ2 but not the latent form.

14. A specific binding member according to claim 3 wherein said human antibody antigen binding domain comprises a VH sequence of the DP50 germ line, or a re-arranged form thereof.

15. A specific binding member according to claim 1 or claim 2 wherein said human antibody antigen binding domain is for the TGF-β isoform TGF-β1.

16. A specific binding member according to claim 15 wherein said human antibody antigen binding domain comprises a VH domain which has the amino acid sequence shown in any of Figure 1(a) (i), Figure 1(a) (ii) and Figure 1(c) (i).

17. A specific binding member according to claim 15 or claim 16 wherein said human antibody antigen binding domain comprises a VL domain which has the amino acid sequence shown in any of Figures 1(b) (i), 1(b) (ii) and 1(a) (iii).

18. A specific binding member according to claim 17 wherein said human antibody antigen binding domain comprises a pairing of a VH domain and a VL domain selected from:

(a) 1B2 VH, of which the amino acid sequence is shown in Figure 1(a) (i), and 7A3 VL, of which the amino acid sequence is shown in Figure 1(b) (i);

(b) 31G9 VH, of which the amino acid sequence is shown in Figure 1(a) (ii), and 31G9 VL, of which the amino acid sequence is shown in Figure 1(a) (iii);

(c) 27C1 VH, of which the amino acid sequence is shown in Figure 1(c) (i), and 10A6 VL, of which the amino acid sequence is shown in Figure 1(b) (ii).

19. A specific binding member according to claim 18 wherein said human antibody antigen binding domain comprises the VH domain 27C1 VH, of which the amino acid sequence is shown in Figure 1(c) (i), and the VL domain 10A6 VL, of which the amino acid sequence is shown in Figure 1(b) (ii).

20. A specific binding member according to claim 15

wherein said human antibody antigen binding domain comprises a VH domain which comprises a CDR3 with an amino acid sequence selected from those shown in Figure 3.

21. A specific binding member according to claim 20 wherein said CDR3 has the sequence shown for CDR3 of 27C1 VH.

22. A specific binding member according to claim 15 wherein said human antibody antigen binding domain is comprises the 31G9 VH domain of which the sequence is shown in Figure 1(a) (ii) and the CS37 VL of which the sequence is shown in Figure 14.

23. A specific binding member according to claim 15 which competes for binding to TGF-β1 with a specific binding member according to claim 18.

24. A specific binding member according to claim 23 which competes for binding to TGF-β1 with a specific binding member according to claim 19.

25. A specific binding member according to claim 15 which competes for binding to TGFβ1 with a specific binding member according to claim 22.

26. A specific binding member according to claim 15

which binds the peptide TQYSKVLSLYNQHN.

27. A specific binding member according to claim 1 wherein said human antibody antigen binding domain is for the TGF-β isoforms TGF-β1 and TGF-β2.

28. A specific binding member according to claim 27 wherein said human antibody antigen binding domain comprise a VL domain with the amino acid sequence shown in Figure 4 and a VH domain with the amino acid sequence shown in Figure 1(a) (ii).

29. A specific binding member according to claim 27 which competes for binding to TGF-β1 and for binding to TGF-β2 with a specific binding member according to claim 28.

30. A specific binding member according to any preceding claim comprising a single-chain Fv antibody molecule.

31. A specific binding member according to any of claims 1 to 29 which comprises one or more amino acids in addition to those forming said human antibody antigen binding domain.

32. A specific binding member according to claim 31 comprising an antibody constant region.

33. A specific binding member according to claim 32 which comprises a whole antibody.

34. A specific binding member according to claim 32 or 33 wherein said antibody constant region is IgG4 isotype.

35. A method comprising causing or allowing binding of a specific binding member according to any preceding claim to TGF-β1 isoform and/or TGF-β2 isoform of human TGF-β.

36. A method according to claim 35 wherein binding takes place *in vitro*.

37. A method according to claim 35 wherein binding takes place *in vivo*.

38. A method according to any of claims 35 to 37 wherein said binding of the specific binding member neutralises said isoform or isoforms.

39. Use of a specific binding member according to any of claims 1 to 34 in the manufacture of a medicament for treating an individual to counteract effects of TGF-β which are deleterious to the individual.

40. Use according to claim 39 wherein said effects

are fibrosis promoting effects.

41. Use according to claim 40 wherein said individual has a condition selected from the group consisting of glomerulonephritis, neural scarring, dermal scarring, ocular scarring, lung fibrosis, arterial injury, proliferative retinopathy, retinal detachment, adult respiratory distress syndrome, liver cirrhosis, post myocardial infarction, post angioplasty restenosis, keloid scarring, scleroderma, vascular disorders, cataract, and glaucoma.

42. Use according to claim 41 wherein said condition is neural scarring or glomerulonephritis.

43. Use according to claim 39 wherein said effects contribute to an immune or inflammatory disease condition.

44. Use according to claim 43 wherein said condition is selected from the group consisting of rheumatoid arthritis, macrophage deficiency disease and macrophage pathogen infection.

45. Nucleic acid encoding a specific binding member according to any of claims 1 to 34.

46. Nucleic acid according to claim 45 which is part

of an expression vector.

47. A method which comprises use of nucleic acid according to claim 45 or claim 46 in an expression system for production of a specific binding member according to any of claims 1 to 29.

48. A host cell containing nucleic acid according to claim 45 or claim 46.

49. A host cell according to claim 48 which is capable of producing said specific binding member under appropriate culture conditions.

50. A method of producing a specific binding member according to any of claims 1 to 34 comprising culturing a host cell according to claim 49 under appropriate conditions for production of said specific binding member.

51. A method according to claim 50 wherein following said production said specific binding member is isolated from the cell culture.

52. A method according to claim 51 wherein following said isolation the specific binding member is used in formulation of a composition comprising at least one additional component.

53. A method according to claim 52 wherein said composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient.

54. A pharmaceutical composition comprising a specific binding member according to any of claims 1 to 34 and a pharmaceutically acceptable excipient.

55. A method of treatment of a condition in which effects of TGF-β are deleterious to an individual, the method comprising administration of a pharmaceutical composition according to claim 54 to the individual.

56. A method according to claim 50 wherein said effects are fibrosis promoting effects.

57. A method according to claim 56 wherein said individual has a condition selected from the group consisting of glomerulonephritis, neural scarring, dermal scarring, ocular scarring, lung fibrosis, arterial injury, proliferative retinopathy, retinal detachment, adult respiratory distress syndrome, liver cirrhosis, post myocardial infarction, post angioplasty restenosis, keloid scarring, scleroderma, vascular disorders, cataract, and glaucoma.

58. A method according to claim 57 wherein said condition is neural scarring or glomerulonephritis.

59. A method according to claim 55 wherein said effects contribute to an immune or inflammatory disease condition.

60. A method according to claim 59 wherein said condition is selected from the group consisting of rheumatoid arthritis, macrophage deficiency disease and macrophage pathogen infection.

The Patent Office

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Claims searched:	1 to 60	**Date of search:**	20 January 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): C3H (HB7P)

Int Cl (Ed.6): CO7K 16/22

Other: ONLINE: WPI, CABS, EMBASE, CEABA, DBA, CBA

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
Y	EP 0290012 A1	(ONCOGEN) see page 4 lines 55 to 58 Claims 15 to 17.	Claim 1 at least
Y	WO 95/26203 A1	(UNIVERSITY OF MANCHESTER) see page 1 lines 1 to 13, page 4 line 18 to page 5 line 3 and the example.	Claim 1 at least
Y	WO 93/11236 A1	(MRC & CAMBRIDGE ANTIBODY TECHNOLOGY) see page 1 line 3 to page 2 line 11 and page 27 lines 11 to 25.	Claim 1 at least
Y	Nature, Vol. 346, 26th July 1990, Border *et al*, "*Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1*", pages 371 to 374		Claim 1 at least

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.